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Effect of Malaria on HIV Viral Load and Maternal Outcomes in HIV-positive Pregnant women of Accra, Ghana

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EFFECT OF MALARIA ON HIV VIRAL LOAD AND MATERNAL OUTCOMES IN HIV-POSITIVE PREGNANT WOMEN OF ACCRA, GHANA

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HIV-1 positive pregnant women co-infected with malaria face increased risk of Mother-to-child-transmission (MTCT) of HIV. Placental malaria has been implicated, whether through an increase in placental or peripheral HIV viral load or a disruption of placental architecture. But the mechanism is not well understood. There are no studies in West Africa, where HIV and malaria co-infection is prevalent.

We determined whether reported malaria infection in HIV-1 positive pregnant women affects either peripheral or placental HIV viral load. Establishing how malaria affects HIV-positive pregnancies in this population is of public health significance.

130 HIV-positive pregnant women in their third trimester were recruited at the Korle-Bu Teaching Hospital in Accra, Ghana from 2007 to 2009 and followed prospectively until delivery. Of 130 recruited, 65 delivered at Korle-Bu. HIV-1 RNA concentration of peripheral and placental blood samples were measured using Amplicor HIV-1 Monitor version 1.5. Giemsa-stained peripheral and placental thick blood films were analyzed for malaria parasitemia. Periumbilical and peripheral full-thickness placental tissue biopsies were fixed, stained with Giemsa and H&E, and assessed by a pathologist blinded to subject data.

The overall mean peripheral and placental HIV-1 viral loads were 5045 copies/ml (SD 16014) and 3389 copies/ml (SD 10679), respectively. No significant effect was seen between subjects reporting and not reporting malaria during pregnancy. Significance was seen in the effect of 2 or more doses of intermittent preventative treatment on reducing incidence of malaria ($p<0.0001$) and in the effect of combination antiretroviral therapy in reducing HIV viral load compared to single-dose NVP at delivery ($p=0.05$).

The low mean HIV-1 viral loads in this population may be responsible for the low rate MTCT at Korle-Bu (0.3 percent, unpublished data). The low rate of transmission warrants further research on the local placental factors influencing transmission. Aggressive antimalarial and antiretroviral therapies are having a significant impact on reducing risk of developing clinical malaria and risks of MTCT of HIV, but better widespread access to care is still needed.

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"In much of the world, the most dangerous thing a woman can do is become pregnant."

-- *Nicholas Kristof,* Half the Sky

I. INTRODUCTION

Maternal morbidity and mortality continue to be one of the most important global health issues, particularly in sub-Saharan Africa, home to half of all maternal deaths. According to the World Health Organization (WHO), 19 of the 20 countries ranked highest in maternal mortality ratios are in sub-Saharan Africa, where the maternal mortality rate averages between 920 to 1050 per 100,000 live births.[1,2] In underdeveloped nations, maternal mortality rates have changed very little in the past 15 years and only about 33% of all maternal deaths are prevented. [1] In addition, for every woman who dies from obstetric causes, close to 20 more suffer injury, infection or disease – nearly 10 million women each year. [2] But women are not the only population that suffers from high maternal death rates. Motherless children in the developing world are ten times more likely to die within the first two years of life. [2] Social development and well-being also suffer. The old African saying, "If you take all the men out of a village, nothing will change; if you take all the women out, the village will not survive," applies. Women are the backbone of society in sub-Saharan Africa, rearing the children, managing the household, feeding the village. Many believe that the women of the developing world could play a major role in ending the cycle of poverty. [49] It is, therefore, of paramount importance to reduce maternal morbidity and mortality on the African continent -- for women, children, and sustainability of most African society.

Within the 60% of preventable deaths in sub-Saharan Africa, there are five

complications that account for over 70% of all maternal morbidity. [2] Hemorrhage continues to be the leading cause of mortality in the developing world, accounting for 25% of all maternal deaths and up to 46% in some populations in West Africa. [4] Other causes include unsafe abortion (13%), eclampsia (12%), and obstructed labor (8%). A contributing factor to all these causes is, of course, access to care. Better access to care would reduce deaths due to obstructed labor and eclampsia. Better resources could prevent so many fatal maternal hemorrhages.

Complications from infectious diseases during pregnancy is the second leading cause of maternal mortality in sub-Saharan Africa, accounting for nearly 15% of all maternal deaths. [4] The HIV pandemic continues to devastate this region, accounting for more than 70% of the world's 40 million HIV-1 infected individuals. [7] And, like maternal mortality, this region accounts for a disproportionate number of the world's HIV infected women, more than 80%.[3]

But though HIV is more notorious, malaria remains the most prevalent infectious disease affecting sub-Saharan Africa, and the most preventable. [2] In the adult population, women are at higher risk of malaria infection than men, and pregnant women are at more risk than non-pregnant women.[20] Up to 66% of pregnant women in sub-Saharan Africa are infected with malaria.[17] Thus, it is clear that both diseases significantly increase risk of maternal morbidity and mortality. But the effect of co-infection of the two diseases is still largely unknown.

A. Overview of HIV in Pregnancy

Among pregnant women in sub-Saharan Africa, HIV prevalence rates can exceed 40%.[3] The most significant risk of an HIV-positive pregnancy is the risk to the future

child, specifically risk of mother-to-child transmission of the HIV virus. Mother-to-child transmission (MTCT) of HIV-1 continues to be a major global health issue, particularly in resource-limited countries such as those of sub-Saharan Africa. According to WHO statistics, an estimated 420,000 children were infected with HIV-1 in 2007, over 90% of which acquired via MTCT.[2,4] HIV-infected infants have a higher risk of mortality than HIV-uninfected infants, but even uninfected infants who were exposed to HIV have a much higher risk of mortality than infants unexposed. [50] Reducing HIV infection in women of the developing world is a complex, multifaceted undertaking with mixed success. Reducing risks of MTCT, on the other hand, can be highly successful.

Vertical transmission of HIV can occur in the prenatal, intrapartum, or postpartum period, but 50 to 80% of all transmissions are thought to occur during the time period near or during delivery.[6, 51] Four main factors contribute to the risk of MTCT: 1) high maternal viral load of HIV RNA at time of delivery, 2) prolonged exposure to vaginal secretions during prolonged labor, 3) micro-disruption of placental integrity leading to exposure of fetus to maternal blood, and 4) transmission of HIV through breast milk (postpartum risk). [51] Interventions specifically targeted to each of these four factors have resulted in a dramatic reduction of MTCT.

Antiretroviral therapy has been the cornerstone of reducing maternal HIV RNA viral load, the single most important risk factor for MTCT. In 1994, the Pediatric AIDS Clinical Trials Group (PACTG) protocol 076 demonstrated that a three-part regimen of zidovudine (AZT) given during the prenatal and antepartum period (as well as to the neonate) resulted in a 67% decrease in rate of MTCT. [51,52] The regimen begins at 14 to 34 weeks gestation and continues throughout the pregnancy, with a boost of intravenous

AZT during labor until delivery. [52] The goal is to reduce HIV RNA viral load to undetectable levels, or at minimum, to below 1000 copies/mL. Studies have shown that HIV RNA viral loads above this cut-off carry a 12-fold increased risk of vertical transmission. [53]

For women with viral loads above 1000 copies/mL, elective caesarian sections have been shown to reduce MTCT of HIV. One study found that elective caesarians in women with >1000 copies/mL HIV RNA reduced the risk of MTCT by 90% compared to vaginal delivery or emergency caesarian. [53] Elective caesarian sections reduce the risk of prolonged labor through decreased exposure of fetus to vaginal secretions in the birth canal and decreased microtransmission of maternal blood during uterine contractions. Elective caesarians also reduce the risk of chorioamnionitis, a result of prolonged labor and another factor that may increase risk of MTCT of HIV. [51]

Lastly, avoidance of breastfeeding eliminates the final risk of vertical transmission of HIV to the child. In the developed world, resources exist to supply new mothers who are HIV-positive with replacement formula, but in the developing world, these resources are scarce or financially infeasible. In Ghana, for example, the rate of exclusive breastfeeding in HIV-positive mothers is more than 40%, according to National Data.[16] The risk of transmitting HIV is weighed against the risk of malnutrition to or starvation of the infant, and in most developing nations, mothers risk HIV transmission in order to feed their new child. Though this topic is of great global significance, as avoidance of breastfeeding is the most preventable risk factor associated with MTCT, it will not be discussed here.

Implementation of the above protocols have reduced the rate of MTCT in the

developed world to less than 2%.[3] In 2007, for example, only 67 children were born with HIV in the United States. [54] In the developing world, however, these interventions are difficult to achieve. Without intervention, rates of MTCT can reach 45%. [3] In sub-Saharan Africa, for example, most pregnant women do not have access to prenatal care or counseling, and availability of highly active antiretroviral therapy (HAART) and caesarian delivery is limited. In areas with poor medical infrastructure, the three-part AZT regimen is too expensive and complex for implementation. Abbreviated anti-retroviral regimens (ARV), on the other hand, have been more successful. These simpler and less expensive regimens have been shown to effectively reduce perinatal transmissions in resource-limited countries, significantly reducing the maternal viral load at delivery and therefore decreasing the risk of transmission. [5, 10-14] There are different permutations of regimens, but the most popular regimen is single-dose nevirapine (NVP) administered to the mother at the onset of labor and to the infant between 48-72 hours of life.[2] This is not the most efficacious regimen, but it is the most cost-effective and feasible.[50] Even then, it is estimated that only 33% of HIV-positive pregnant women receive any antiretroviral treatment, suggesting that increased access to care would have a profound effect on reducing MTCT worldwide.[2]

For areas that can afford it, first line therapy should be the additional administration of AZT and lamivudine from 28 weeks gestation to delivery and administration of a one-week course of AZT to the infant. Lamivudine is added to reduce risk of HIV resistance to single-dose NVP. [50] Interestingly, HIV resistance does not appear to develop in subsequent pregnancies to single-dose NVP. In addition, the serious side effect of hepatotoxicity with nevirapine use has not been observed in women receiving only

single-dose nevirapine during labor for prevention of mother to child transmission. [55] Therefore, in the developing world, the benefit of using single-dose NVP far outweighs the risks.

It must also be mentioned that the first step to effectively reducing MTCT rates of HIV in any population is successful identification of pregnant women infected with HIV. This requires widespread and targeted prenatal testing and counseling to all pregnant women through prevention of mother-to-child transmission (PMTCT) programs. With effective programs and basic resources available to identified HIV-positive pregnant women, low rates of MTCT can be achieved, even in developing nations.[54] Reducing risk factors to MTCT is the goal of every obstetrician, but recently a new risk factor has been identified that requires even further intervention, a risk factor that affects nearly 50 million pregnant women on the African continent.[2] That risk factor is malaria.

B. Overview of Malaria in Pregnancy

Though more focus has been placed on the HIV epidemic in sub-Saharan Africa, malaria is also recognized as a major global health issue. There were approximately 881,000 malaria deaths in 2006, of which 91% were in Africa and 85% were children under 5 years of age.[54] It is estimated that 10,000 women and 200,000 infants die each year as a result of malaria infection during pregnancy.[54] Malaria, predominately the *Plasmodium Falciparum* species, can lead to severe maternal anemia during pregnancy, prematurity, and low birth weight, all of which contribute to infant mortality in sub-Saharan Africa.[54] Parasitemia peaks in the second trimester in both primigravidas and multigravidas, but the increased risk for pregnancy-associated malaria (PAM) persists for 60 days postpartum.[20] Thus, malaria during pregnancy is of great global significance,

adversely affecting maternal and neonatal outcomes.

As stated before, pregnant women have the highest risk of developing malaria symptoms than any other adult population in sub-Saharan Africa.[20] The reason for this is one of the unique features of the *P. Falciparum* malaria parasite. Pregnancy selects for a specific population of parasites that express variant surface antigens (VSA).[21] These antigens adhere parasite-infected erythrocytes to chondroitin sulfate A (CSA) on the syncytiotrophoblast lining in the intervillous spaces of the placenta. The process of selection appears to be mediated by upregulated transcription of the var2csa gene. [21] Adherence of parasite-infected erythrocytes to the placental lining leads to sequestration of leukocytes and a local inflammatory response, mediated by numerous cytokines and transcription factors, which will be discussed in the next section.[22] This response can cause a disruption of placental architecture through local necrosis and syncytial degradation, and thus increase the risk of low-birth weight infants and possible fetal infection through breakdown of placental architecture.[23] In an woman co-infected with HIV, this could possibly lead to microtransmission of maternal blood and entry of the virus.

Multiple studies have established that primigravidas are most at risk for malaria, with prevalence decreasing with increasing gravity.[18,19] This is particularly true in malaria endemic countries, where women have an overall high immunity to malaria but have not developed specific antibodies to placental antigens until after their first pregnancy. The specific antibodies that develop are anti-VSA IgG immunoglobulins, and they have a protective effect against placental malaria during subsequent pregnancies. These antibodies prevent cytoadherence of the infected erythrocytes to the placenta, and thus

reduce the risk of placental destruction. [21] In women with HIV, however, the reduced immunity may nullify the ability of the body to mount a response against the malaria parasite with anti-VSA IgG.[33, 37] Interestingly, though, one recent study demonstrated that decreased levels of anti-VSA IgG were not associated with increased rates of clinical malaria or adverse maternal or infant outcomes in HIV-positive pregnant women, suggesting that several other mechanisms are at play.[56] It seems clear that the interaction of malaria and HIV in co-infected pregnant women of sub-Saharan Africa is a complex process that is not entirely understood.

C. Co-infection of HIV and Malaria in Pregnancy

In 2004, the WHO identified the prevention of malaria during pregnancy as a necessary tool in limiting maternal morbidity and mortality, particularly in HIV-positive individuals.[2] The relationship between malaria and HIV was known beforehand, as several studies suggest that the interplay of the two infections seems to fuel the continued spread of both diseases. [24-30] HIV diminishes immunity thereby increasing the risk of malaria infection and the development of clinical malaria.[24-26] Conversely, malaria has been show to increase HIV replication by activating the immune system by mechanisms similar to that found with vaccination and bacterial infections.[27-30] The interplay of the two infectious diseases is also clearly seen during pregnancy. One study demonstrated that HIV-1 positive pregnant women with peripheral malaria parasitemia had a two-fold higher HIV-1 plasma concentration than those HIV-positive pregnant women without malaria infection, when matched for initial HIV-1 viral load prior to infection.[31] Other studies have demonstrated that co-infection of HIV and malaria is associated with an increased risk of higher parasitemias, maternal death, postpartum maternal anemia, and

severe clinical disease compared to HIV-negative women. [37, 57]. Placental and clinical malaria are more common in HIV-positive women, as are cases of infant and maternal death. [3,32] Recently, one additional parameter to the relationship between HIV and malaria in pregnancy has emerged: the potential risk of increasing MTCT.

The studies addressing the effect of malaria parasitemia on MTCT of HIV are, unfortunately, conflicting. The relationship was first identified in a study in Cameroon, where researchers found higher MTCT rates correlated with peak rainy seasons and with higher malaria transmission, suggesting an association between MTCT and higher rates of malaria during pregnancy.[3] In Uganda, researchers found a significant increase in vertical HIV transmission associated with placental malaria. [34,35] However, a study in Mombasa, Kenya found no impact of placental malaria on MTCT.[36] And lastly, a study in Kisumu, Kenya found an increased risk of MTCT only at high maternal parasitic densities compared with low parasitic densities.[37] It has been proposed that significant discrepancies in methodological approaches to defining placental malaria in the Kenya studies can account for differences in outcome, but further studies are needed to confirm the role of malaria infection on HIV-1 vertical transmission and the mechanisms at play.

As discussed before, the interaction of malaria and HIV in pregnancy is complex, particularly at the level of the placenta. Two main mechanisms have been proposed to explain the risk of placental malaria on MTCT of HIV: 1) increased HIV-1 viral load and 2) disruption of placental architecture. The first involves the relationship between malaria infection and increased HIV RNA viral replication. Malarial infection results in a temporary increase in HIV RNA, an increase that will return to baseline after two months of antimalarial treatment. [27, 41] However, a transient rise in HIV RNA viral load during

the last trimester could increase the risk of MTCT. [27,3,34] But a recent analysis by Brahmbhatt et al demonstrated that even after adjusting for maternal HIV viral load, placental malaria significantly increased the risk of MTCT, suggesting that increased HIV-1 viral concentration is not the sole mechanism for transmission.[35]

As mentioned previously, numerous cytokines and transcription factors mediate the local inflammatory response induced by sequestration of malaria in the placenta. Thus, it is possible that these factors could increase risk of MTCT by affecting the local placental environment, but little data is available and the studies that have been done are heterogenous. For example, in vitro studies of placental co-infection of HIV-1 and malaria have shown dose-dependent increases in local HIV-1 viral replication related to increased expression of TNF-alpha, a pro-inflammatory cytokine.[32] However, another study found significantly reduced placental viral load compared to peripheral viral load among women with confirmed placental malaria, despite high levels of TNF-alpha.[38] Other studies have identified cytokines and factors induced by placental malaria presence with a more protective role against MTCT. One study suggests that placental malaria induces an upregulation of Th1 cytokine interferon-gamma in the intervillous blood mononuclear cells, which are known to reduce HIV replication.[37] Leukemia inhibitory factor, which inhibits HIV replication, has been shown to be upregulated in women with placental malaria who do not transmit vertically.[58] Studies have also shown that placental malaria can increase expression of macrophage inflammatory protein 1-Beta, which blocks HIV entry into cells.[59]

Thus, it appears that there is much controversy surrounding both proposed mechanisms for the effect of placental malaria on MTCT of HIV. Does placental malaria

protect against MTCT or promote it? Are there cytokine or immune targets we can use to reduce the risk of vertical transmission of HIV? Can we identify specific placental immune factors in HIV-positive women who do not transmit? Do these same factors lead to adverse maternal outcomes? Understanding the mechanism by which HIV and malaria interact is incredibly important to answering these questions.

D. Prevention of Malaria in Pregnancy

Due to the high risk of maternal morbidity and infant mortality associated with pregnancy-associated malaria, the WHO identified malaria prevention as an essential component of prenatal care among all pregnant women in areas of stable *P. falciparum* transmission in Africa.[39,40,54] Specifically, the WHO recommends use of insecticide-treated bednets (ITN) and intermittent preventive treatment (IPT) during the course of the pregnancy. A curative dose of sulfadoxine-pyrimethamine (SP, Fansidar) administered twice at monthly intervals after the first trimester is the preferred method of prophylaxis for HIV-negative pregnant women.[54] Though toxicity due to the sulfa component can occur, SP's low cost, relative safety, ease of use, and efficacy outweigh adverse effects.[39] This two-dose regimen of IPT with sulfadoxine-pyrimethamine significantly reduced the incidence of placental malaria, LBW, and maternal anemia in women having a first or second pregnancy, even in areas where the prevalence of drug resistance was 19 to 26 percent.[41]

For HIV-positive pregnant women, at least three or more doses of IPT is needed to prevent placental malaria.[2] Numerous studies showed that while two doses of IPT with sulfadoxine-pyrimethamine was sufficient for HIV-negative women, more frequent dosing was needed in HIV-positive women to achieve the same effect.[41] Monthly IPT

dosing was significantly more effective than the two-dose regimen, as supported by a lower rate of placental malaria and an increase in birth weight, even in areas where the prevalence of drug resistance was as high as 39 percent. In HIV-negative women, monthly dosing did not improve outcomes over the two-dose regimen.[41]

The more aggressive IPT regimens required for HIV-positive pregnant women supports the unique relationship between HIV and malaria co-infection during pregnancy, and the need to understand that relationship. In addition the scientific implications, understanding this relationship has significant policy implications towards the development of additional targeted, cost-effective strategies to reduce adverse maternal and infant outcomes. Are more funding and efforts needed to prevent placental malaria infection in HIV-positive pregnant women or will more access to ARV treatments have a greater effect? In resource-limited environments, these are highly important questions that need to be answered.

E. Overview of Ghana

No previous study has investigated the relationship of HIV and malaria co-infection among pregnant women of Accra, Ghana. The Republic of Ghana is located in West Africa on the Coast of Guinea with a population of almost 24 million people. Accra is the capital of Ghana and has a population of about 3 million people. Compared to the rest of sub-Saharan Africa, HIV/AIDS rates in Ghana have remained relatively low and stable, rising from an estimated 2.4% in 1992 to 3.1% in 2004.[16] In 2006, government-sponsored programs such as HIV testing and counseling sites and Prevention of Mother-to-Child Transmission (PMTCT) sites were increased in number throughout the country, including at Korle-Bu Teaching Hospital in Accra, Ghana. This reduced the national HIV

prevalence rate from 3.1% in 2004 to 1.9% in 2007. Among pregnant women, the 2007 prevalence rate of HIV was slightly higher at 2.6% on average, 3.4% in urban locations, and there were 3,000 new infections among children in 2007.[16]

Ghana is holoendemic for malaria and has a stable rate of malaria transmission. It is perennial but peaks during the rainy and post-rainy season (June to August). *P. falciparum* causes over 98% of all malaria infections.

The low prevalence rate of HIV but stable rate of malaria transmission make Ghana an interesting comparison study to the previous studies on malaria and MTCT in Eastern and Southern Africa, where HIV prevalence rates can reach up to 20% in some areas. [34-37] The location of the study at the Korle-Bu Teaching Hospital is also an interesting comparison to the rural environments of most of the previous studies. Korle-Bu is a tertiary hospital and the largest teaching hospital in Western Africa. The hospital has 1600 inpatient beds and several thousand outpatient visits a year. It also serves as the primary teaching hospital for University of Ghana Medical School. It is the referral center for all high-risk pregnancies, complicated pregnancies (including gestational diabetes, hypertension in pregnancy, or other comorbid diseases), and pregnancies in the third trimester in the Greater Accra and Eastern Region. Thus, it is the highest level of care available for the average Ghanaian woman who becomes pregnant in these areas.

II. STATEMENT OF PURPOSE

The first specific aim (1) was to determine the prevalence of *P. falciparum* parasitemia in peripheral blood of HIV-1 negative and positive pregnant women attending antenatal clinic (ANC) at Korle-Bu Teaching Hospital in Accra, Ghana. It was

our hypothesis that HIV-1 positive women would have higher incidence of malaria parasitemia in comparison to HIV-1 negative women during pregnancy.

The second specific aim was two-fold. One aspect of that aim (2a) was to compare the intensity of *P. falciparum* parasitemia in the peripheral blood and placenta of HIV-1 positive women with or without clinical malaria at delivery. It was our hypothesis that there would be a high density of parasitemia in both blood and placenta, especially in women with clinical malaria. The other aspect of the second aim (2b) was to determine if placental *P. falciparum* parasitemia was associated with increased peripheral HIV-1 RNA concentration in HIV-1 positive women at delivery. It was our hypothesis that HIV-positive women with increased intensity of placental parasitemia would also have higher HIV-1 RNA concentration in the peripheral blood.

The third specific aim (3) was to determine the incidence of HIV-positive infants born to positive HIV-1 women with or without acute malaria infection. It was our hypothesis that HIV-positive women with placental malaria would have a higher incidence of HIV-1 positive infants.

III. METHODS

The project was a two-year pilot study to investigate the specific aims listed above. Initial approval for the project was obtained through the Yale Institutional Review Board (IRB) and the Ghana IRB.

There were four parts to the pilot study design. Part I involved initial recruitment of both HIV-negative and HIV-positive subjects and the obtainment of demographic and specific disease-related information as reported by the subjects in a questionnaire. Part I also involved the obtainment of blood samples from the HIV-positive subjects at

recruitment for thick blood smear, CD4 and viral load assessment. Part II involved the postpartum period - the obtainment of information relating to delivery, blood samples from the mother for thick smear and viral load assessment, and collection and processing of the placentas from HIV-positive subjects. Part III was placental histopathological analysis. Part IV was assessment of MTCT of HIV in the research population based on HIV-DNA testing of infant blood samples.

A. Background

The study was conducted at Korle-Bu Teaching Hospital in Accra, Ghana in five different departments. Initial recruitment of the HIV-positive pregnant women occurred at The Fevers Unit, the medical ward for patients with HIV, tuberculosis and other infectious diseases. All patients who are treated at Korle-Bu and who test positive for HIV-1 are referred to this department, including all HIV-1 positive pregnant women. Further recruitment of HIV-positive pregnant women occurred in the Maternity Wards of the OB-GYN department. Recruitment of HIV-negative pregnant women occurred at the Antenatal Center (ANC) of the OB-GYN department. All pregnant women receiving care at Korle-Bu's ANC are given a purple maternity booklet to carry with them at all times. This booklet serves as the obstetric chart - documenting routine prenatal care, medication use (including IPT administration), results of screening tests, and any medical complications that occur during the pregnancy. Demographic and disease-specific information was obtained from this booklet. The National Health Scheme, Ghana's government healthcare system, provides free medical care for all pregnant patients, including antiretroviral and antimalarial medications.

All women who have positive pregnancy tests and who are referred to Korle-Bu

participate in the Prevention of Mother to Child Transmission (PMTCT) program. Pregnant women are encouraged to obtain HIV testing and over 98% of pregnant women agree to be tested (data not presented). If women test positive for HIV, they are referred to the Fevers Unit to begin HIV care. At the Fevers Unit, blood samples are taken to determine CD4 count.

B. Consent

Two separate written consent forms were devised - one describing the procedure for HIV-negative subjects, one describing the procedure for HIV-positive subjects. The consent was written in English. If the subject did not speak English, a translator was employed to explain the details of the consent. All subjects received a written copy of the consent form and were asked to sign a separate form confirming that consent was obtained. If subjects were unable to write, they provided their fingerprint, a common practice among illiterate individuals in Ghana.

C. The Questionnaire

A brief questionnaire was designed to obtain basic information regarding obstetric history (past and present), socio-economic history, demographics, and disease-specific history of both HIV and malaria exposure. The questionnaire was written in English. As with the consent, if the subject did not speak English, a translator was employed.

D. Inclusion/Exclusion Criteria

All HIV-positive pregnant women receiving care at the Korle-Bu Fevers Unit and Maternity Ward were eligible. Women were ineligible if they were part of other research studies, less than 18 years of age, or had hypertension or complications during pregnancy.

E. Implementation

Part I: Recruitment

130 HIV-positive pregnant women in their second or third trimester who were eligible were recruited into the study at the Fevers Unit of Korle-Bu Teaching Hospital in Accra, Ghana from October 2007 to May 2008 and then again from September 2008 to April 2009. 57 subjects were recruited in the 2007-08 period and 74 were recruited in the 2008-09 period.

During the recruitment phase, the subjects were verbally consented and asked a series of questions detailed in a questionnaire, utilizing a translator if necessary. Careful documentation was made of current or prior treatment and time of diagnosis for both HIV and malaria. Blood samples were obtained for initial viral load and CD4 count, and thick blood films were prepared for malaria parasitemia analysis. Finally, a white sticker with the research cell phone number and instructions to call if there were any questions was placed on the maternity folder. The subjects were also encouraged to call when they went into labor so that the research team could obtain the necessary samples and information at delivery. The subjects were then followed prospectively until delivery.

124 documented HIV-seronegative pregnant women matched for age (+/-5 years), gravity (1,2,3+), parity (0,1,2+), and gestational age (+/- 2 weeks) were recruited at the Maternity Ward while waiting for their prenatal appointment. The HIV-negative subjects were consented in the same fashion as the HIV-positive subjects and asked to answer the same questionnaire. No blood samples were obtained, and the HIV-negative subjects were not followed until delivery.

Part II: Delivery

When an HIV-positive subject had delivered, 3 blood samples were collected

within 48 hours of delivery - one maternal peripheral blood sample for peripheral HIV-1 RNA viral load testing, one maternal peripheral thick film preparation for peripheral malaria parasitemia analysis, and one blood sample from the infant for HIV-1 DNA testing.

It was common to recruit subjects into the study at the time of delivery as several HIV-positive pregnant women had been missed in the initial recruitment phase at the Fevers Unit. If the subject had not been recruited prior to delivery, consent was obtained and the questionnaire was given to obtain retrospective data. If the subject had been previously recruited, follow-up questions regarding antiretroviral initiation, malaria exposure and treatment, and additional IPT doses since recruitment were recorded.

All HIV-positive women delivering at Korle-Bu Hospital received a dose of Nevirapine at the onset of labor. All infants born to HIV-positive women at Korle-Bu also received a dose of Nevirapine within 72 hours of birth. According to 2003 WHO guidelines on prophylaxis of HIV in infants born to HIV-positive mothers, infants would receive additional antiretroviral combination therapy (Combivir) for one week if the mother did not start antiretrovirals before 28 weeks gestation, and for 4 weeks if she did not receive any antiretroviral therapy prior to delivery. [2]

Part IIIa: Placental Processing and Examination

At delivery, the placenta was obtained and processed within 48 hours. Periumbilical and peripheral full-thickness biopsies and membrane samples were taken, placed into pencil-labeled cassettes, and fixed in formalin. The formalin-fixed, paraffin-embedded placental disc tissue, including membranes and umbilical tissue, were then sectioned for hematoxylin and eosin staining (H&E). Giemsa staining

immunohistochemistry was also performed on the placental disc according to the procedure outlined by Rogerson et al.[42]

Placenta architecture and malaria parasite presence were assessed by a pathologist blinded to subject data. Presence of *P. falciparum*-infected erythrocytes and of malaria pigment in fibrin and monocytes was noted. Using a systematic method, 500 intervillous blood cells were counted under oil immersion, and classified as uninfected erythrocytes (UE), infected erythrocytes (IE), or leukocytes, subdivided by morphology into lymphocytes, polymorphs, or monocyte-macrophages. Histologic percentage parasite density was defined as IE/(UE + IE), × 100%. [42] Of leukocyte types, only monocyte counts varied between infected and uninfected placentas. A high prevalence of formalin pigment precluded assessment of pigment that was due to chronic or past malaria infection. Malaria quantification was performed by parasitologists at The Noguchi Institute, a prominent research facility located 45 minutes away from the Korle-Bu campus. Parasite densities were calculated by assessing immunohistochemistry placental slides at 203 magnification. Five random fields, each of 1 square mm of the placental slides, were averaged to obtain density of parasites, expressed as number mm2.[42]

Part IIIb: Blood Sampling and Viral Load Assessment

Maternal blood samples were obtained and collected into EDTA tubes. Infant blood samples were obtained in the same manner. CD4 assessment was performed at the Central Lab of Korle-Bu, through the Fevers Unit. HIV-1 RNA concentration of maternal peripheral and placental blood samples were measured using Amplicor HIV-1 Monitor version 1.5 (Roche Diagnostics, Branchburg, New Jersey, USA) with a quantification limit of 400 copies per milliliter.

Part IV: HIV-1 DNA testing of infant samples

HIV-1 DNA testing of the infant were done using Amplicor HIV-1 Monitor version 1.5 (Roche Diagnostics, Branchburg, New Jersey, USA). An infant would be considered HIV-1 positive and the mother a transmitter if HIV-1 DNA PCR testing was positive within 48 hours of delivery, reflecting intrauterine and intrapartum transmission.

Thick Film Preparation and Analysis

Thick films were prepared from a single drop of blood evenly dispersed in a 2cm-diameter circle on a microscope slide using a needle tip, then dried overnight in a sealed container and stained the next day. Both peripheral and placental thick blood films were stained with 20% Giemsa for 10 minutes and examined under oil immersion for malaria parasites. The Giemsa was prepared and films stained at the Microbiology Department of Korle-Bu. The thick films were then analyzed for malaria parasitemia by two separate parasitologists, one at Korle-Bu and one at Noguchi. A thick film was considered negative if 100 microscopic fields showed no parasites.

F. Statistical Methods

Specific aims 1 and 2a were evaluated using descriptive statistics and chi square tests. Logistic regression was used to determine if placental *P. falciparum* parasitemia was associated with increased peripheral and/or placental HIV-1 RNA concentration in HIV-positive women at delivery (Specific Aim 2b). In the absence of data from microscopy or histopathology identifying peripheral or placental malaria parasitemia, reported data from the questionnaire of specific aim 1 was utilized as a surrogate. Paired, one and two-tailed student t-test statistics were performed on the data to determine differences in HIV-1 RNA viral loads among groups reporting and not

reporting malaria. The data analyses were conducted using SPSS version 15.0 (SPSS Inc. 2006).

G. Research Personnel

My purpose in the project was to continue the work begun by the previous Doris Duke Fellow, fine tune the protocol, increase recruitment numbers, conclude the pilot study, analyze the data, and assess further areas of research for future collaborations between Korle-Bu Teaching Hospital and Yale School of Medicine. I recruited 74 HIV-positive pregnant women and 84 HIV-negative pregnant women into the study from October 2008 to April 2009. Of the HIV-positive subjects, I personally collected and processed 32 placentas, 45 maternal peripheral blood samples at delivery, and 15 infant blood samples. I improved the attrition rate of placentas from 40% to 15% by the end of the research period. I also made thick films at recruitment and delivery for all HIV-positive subjects that I recruited. I ran the maternal HIV-1 RNA viral load kits for the available samples and sampled the placentas of the HIV-positive subjects. I was responsible for the day-to-day implementation of the protocol of the pilot study from 2008-09 and for obtaining cooperation from the staff members of the different departments. Finally, I performed the statistical analyses.

Viral processing was conducted at the Virology Lab with the assistance of that department. Placental processing took place in the Pathology department with the assistance of that department. Staining and reading of thick film slides for malaria parasitemia took place in the Microbiology department and the Noguchi Institute of University of Ghana, with the assistance of those two departments.

IV. RESULTS

Part I and Part II of the research project were successfully completed at the conclusion of the two-year pilot study. Processing and analysis of data pertaining to Part III and Part IV have not yet been completed.

A total of 130 HIV-positive subjects were enrolled in the study. 79 delivered at Korle-Bu, leaving 51 lost to follow-up (39%). Of the 79 who delivered at Korle-Bu, peripheral blood samples were obtained for 60 (76%), placental data was available for 25 (32%), and infant blood samples were collected for 15 (19%).

Table 1 describes the demographic characteristics of the HIV-positive and HIV-negative subjects. In comparison to their HIV-positive counterparts, the HIV-negative subjects were twice as likely to be educated at high school level or beyond (51% vs 26%), own their own home (36% vs 19%), work in a professional or office setting (24% vs 9%), use a flush toilet versus an outside latrine or public toilet (45% vs 23%), and have a faucet in the house for water (74% vs 48%).

Table 1: Demographic characteristics of Enrolled Subjects, by HIV status

	HIV-1 positive	HIV-1 negative	P-Value
	N=130	N=124	
Age (median)	31 (SD 6)	29 (SD 5)	
Level of Education			
None	21 (16%)	12 (10%)	
Primary	18 (14%)	15 (12%)	
Junior Secondary	55 (42%)	60 (48%)	

	HIV-1 positive	HIV-1 negative	P-Value
Secondary and above	34 (26%)	63 (51%)	0.004
Home			
Own	25 (19%)	45 (36%)	0.02
Rent	78 (60%)	65 (52%)	
Share	20 (15%)	12 (10%)	
Marital Status			
Married	109 (83%)	106 (85%)	
Single	17 (17%)	16 (15%)	
Occupation			
Professional	5 (4%)	11 (9%)	0.05
Office	4 (3%)	13 (10%)	
Manual Labor	96 (74%)	82 (66%)	
Unemployed	19 (15%)	17 (14%)	
Sanitation (%)			
Flush Toilet	20 (23%)	56 (45%)	0.008
Pit Latrine in house	14 (11%)	6 (5%)	
Public Toilet	33 (25%)	22 (18%)	
Pit Latrine outside	43 (33%)	36 (29%)	
Water (%)			
Faucet in home	62 (48%)	92 (74%)	
Outside Home	82 (63%)	20 (16%)	<0.0001

Table 2 describes the characteristics of HIV-positive subjects enrolled in the study. Among the 130 HIV-positive pregnant women, 64% were diagnosed during the current pregnancy. Of the subjects followed until delivery, 60% had started antiretroviral

medication prior to or during the current pregnancy, and 42% received only nevirapine at labor. The average birth weight of infants born to the HIV-positive subjects was 2.88 kg (SD 0.55), and no significant difference in birth weight was found between subjects reporting malaria and subjects not reporting malaria (2.80 vs 2.94, p=0.17). CD4 counts taken within 6 months of delivery also did not differ between subjects reporting malaria and subjects not reporting malaria (408 vs 452, p=0.63). Of the 13% of subjects whose gestational age at delivery was less than 37 weeks, there was no significant difference in subjects reporting or not reporting malaria.

Also of note, 53% of subjects did not know if their husband or partner was also HIV-positive, 18% reported that their husbands or partners had tested negative for HIV, and 26% reported that their husbands or partners had tested positive for HIV.

Table 2a: Characteristics of HIV-positive pregnant women, at enrollment

	Enrolled
	N=130
CD4 Baseline (median)	423
Diagnosis (%)	
During current pregnancy	83 (64%)
Within year of pregnancy	17 (13%)
More than a year before pregnancy	29 (22%)
Antiretroviral (ARV) Use (%)	
NVP at labour	53 (41%)
Combivir	8 (6%)
Combivir and NVP	68 (52%)

	Enrolled
Start of ARVs (%)	
At labour	55 (42%)
At 28 wks gestation or after	35 (27%)
Before 28 wks gestation	12 (9%)
Before pregnancy	27 (21%)
Partner HIV-status (%)	
Unknown	67 (53%)
HIV positive	34 (26%)
HIV negative	23 (18%)
Mode of Delivery (%)	
SVD	43%
Elective Caesarian	46%
Emergency Caesarian	11%
GA at Delivery	
>37 weeks	86%
34-36 weeks	5%
<34 weeks	8%
Subjects receiving NVP at onset of labor	100%
Antiretroviral Prophylaxis of Infants	
NVP	50%
NVP + Combivir	50%

Table 2b: Relationship of Malaria to Delivery Outcomes in HIV-positive subjects

	Enrolled	No Malaria Reported	Reported Malaria	P-Value
	N=130	N=73	N=53	
Birth Weight of Infant (mean) (kg)	2.88 (SD 0.55)	2.94 (SD 0.62)	2.80 (SD 0.50)	0.17
CD4 Baseline (median)	423	408	359	
ARV Use (%)				
NVP at labour	53 (41%)	12 (16%)	22 (42%)	0.0006
Combivir	8 (6%)	7 (10%)	1 (1%)	
Combivir and NVP	68 (52%)	51 (70%)	30 (57%)	
GA at Delivery				
>37 weeks	112 (86%)	62 (85%)	47 (86%)	
34-36 weeks	7 (5%)	4 (6%)	2 (4%)	
<34 weeks	10 (8%)	7 (9%)	4 (7%)	0.37

Table 3 compares malaria characteristics between HIV-positive and HIV-negative subjects. There was no significant difference in reported prevalence of malaria during pregnancy between HIV-positive subjects (41%) and HIV-negative subjects (39%). Among HIV-positive and HIV-negative subjects, there was no significant difference in gravity or parity between subjects reporting malaria and subjects with no malaria reported during their pregnancy. There was, however, a lower percentage of HIV-positive women receiving more than 2 doses of IPT (23%) compared to HIV-negative women receiving 2 or more doses (31%), but this was not statistically significant (p=0.55). Among HIV-positive and HIV-negative groups receiving 2 or more doses of IPT, there was a significant difference between the percent of subjects reporting malaria (70% and 71% respectively) and subjects not reporting malaria (30% and 28% respectively). See Table 3b. Likewise, 13% of HIV-positive women reporting malaria received 2 or more doses of IPT, whereas 60% received no IPT during pregnancy. No difference was noted in gravity

or parity among women reporting malaria and not reporting malaria in either the HIV-positive or HIV-negative populations.

In terms of insecticide-treated bednet (ITN) use, over 75% of both HIV-positive and HIV-negative groups did not utilize ITNs. But, within the HIV-positive group who did use ITNs, 28% reported malaria compared to 72% who did not.

Table 3a: Characteristics of enrolled subjects, based on HIV status and Reported Malaria Exposure

	HIV-1 positive				HIV-1 negative			
	All	No Malaria Reported	Reported Malaria	Hospitalized for Malaria	All	No Malaria Reported	Reported Malaria	Hospitalized for Malaria
	N=130	N=73 (56%)	N=53 (41%)	N=15 (12%)	N=124	N=75 (60%)	N=48 (39%)	N=9 (7%)
Gravity								
Primagravidae	19 (15%)	11 (15%)	8 (15%)	2 (13%)	23 (19%)	13 (17%)	10 (21%)	3 (33%)
Secundigravidae	29 (22%)	21 (29%)	8 (15%)	2 (13%)	22 (18%)	16 (21%)	6 (13%)	1 (11%)
Multigravidae	77 (59%)	41 (56%)	36 (69%)	11 (73%)	78 (63%)	46 (61%)	32 (67%)	5 (56%)
Parity								
Nulliparous	45 (35%)	27 (37%)	18 (36%)	7 (47%)	42 (34%)	26 (35%)	16 (33%)	4 (44%)
Primaparous	27 (21%)	19 (26%)	8 (15%)	3 (20%)	28 (23%)	16 (21%)	12 (25%)	2 (22%)
Multiparous	53 (42%)	27 (37%)	26 (53%)	5 (33%)	53 (43%)	33 (44%)	20 (42%)	3 (33%)
IPT SP Doses								
0	73 (56%)	39 (53%)	33 (62%)	7 (47%)	52 (42%)	32 (43%)	20 (42%)	3 (33%)

	HIV-1 positive				HIV-1 negative			
1	27 (21%)	13 (18%)	13 (25%)	6 (40%)	32 (26%)	15 (20%)	17 (35%)	6 (66%)
≥2	30 (23%)	21 (29%)	9 (17%)	2 (13%)	39 (31%)	28 (37%)	11 (23%)	0
ITN use								
Yes	29 (22%)	21 (29%)	8 (15%)	4 (27%)	15 (12%)	6 (8%)	9 (19%)	3 (33%)
No	94 (78%)	50 (68%)	44 (83%)	10 (67%)	108 (88%)	69 (92%)	39 (81%)	6 (67%)

Table 3b: Number of IPT Doses on Malaria Infection, by HIV status

	IPT SP Doses			P-value
HIV-positive	0	1	2	
No Malaria Reported	39 (53%)	13 (18%)	21 (29%)	
Malaria Reported	33 (62%)	13 (25%)	9 (17%)	<0.0001
HIV-negative				
No Malaria Reported	32 (43%)	15 (20%)	28 (37%)	
Malaria Reported	20 (42%)	17 (35%)	11 (23%)	<0.001
Reported Malaria				
HIV-positive	33 (62%)	13 (25%)	9 (17%)	
HIV-negative	20 (42%)	17 (35%)	11 (23%)	<0.05

	IPT SP Doses			P-value
Hospitalized for Malaria				
HIV-positive	7 (47%)	6 (40%)	2 (13%)	
HIV-negative	3 (33%)	6 (66%)	0	<0.0001

Figure 1: Relationship of IPT uptake (%) on Reported Malaria, by HIV-Status

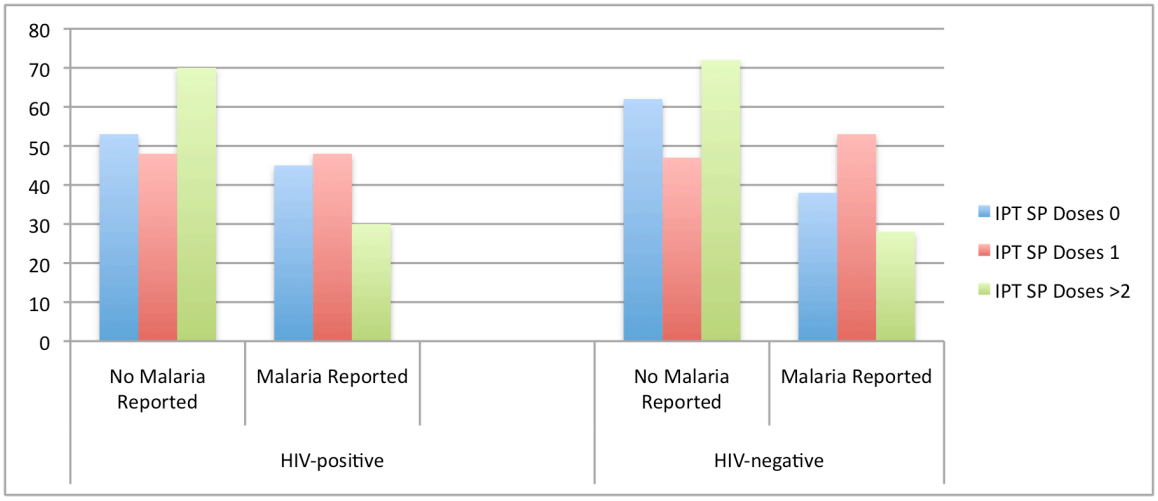

Table 4 shows the relationship between reported malaria infection and HIV-1 RNA viral load, both peripheral and placental. At delivery, the overall mean peripheral HIV-1 viral load was 5045 copies/mL (SD 16014), and mean placental HIV-1 viral load was 3389 copies/mL (SD 10679). There was no significant difference between HIV-1 RNA viral load (either placental or peripheral) in subjects reporting malaria during pregnancy and subjects not reporting malaria.

Table 4: Relationship of Reported Malaria Exposure to HIV-1 Viral Load and Birth Weight

	Delivered	No Malaria Reported	Malaria Reported	P Value
	N=28	N=16	N=12	
HIV-1 Peripheral Viral Load (mean)	5045 copies/ml(SD 16014)	6385 copies/ml(SD 20001)	2914 copies/ml (SD 4476)	0.26
HIV-1 Placental Viral Load (mean)	3390 copies/ml(SD 10679)	3493 copies/ml(SD 12433)	3400 copies/ml (SD 9098)	0.74

Table 5 shows the relationship of ARV treatment during pregnancy to HIV-1 viral load at delivery. 52% of HIV-1 positive women received Combivir and NVP and had significantly lower peripheral HIV-1 viral load than the 41% of women who received NVP only (8979 copies/ml vs 2131 copies/ml, p=*0.05*).

Table 5: Relationship of ARV during pregnancy to HIV-1 Viral Load at Delivery

	NVP only (41%)	Combivir and NVP (52%)	P value
HIV-1 Peripheral Viral Load (mean)	8979 copies/ml (SD 22822)	2131 copies/ml (SD 7268)	0.05
HIV-1 Placental Viral Load (mean)	4154 copies/ml (SD13940)	2956 copies/ml (SD 8552)	0.36

Information on malaria parasitemia as determined by microscopy for maternal

peripheral and placental thick blood films and histopathological analysis of placental samples are not available at this time.

V. DISCUSSION

This two-year pilot study investigating the role of malaria on HIV-positive pregnancy outcomes in Accra, Ghana suggests that, overall, malaria exposure during pregnancy does not exacerbate HIV in this population. HIV-1 RNA viral loads, both peripheral and placental, were not significantly different between HIV-positive subjects who reported malaria infection and who did not report malaria infection (2914 copies/mL, 3400 copies/mL vs 6385 copies/mL, 3493 copies/mL, p=0.26, 0.74). Prevalence rates of reported malaria among HIV-positive and HIV-negative subjects were equivalent (41% and 39%), and though almost twice as many HIV-positive subjects reported hospitalization for malaria (12% vs 7%), this was not statistically significant (p=0.22). But though no significant relationship between malaria infection and maternal HIV-1 RNA viral load has been established by this study, there are two important findings that may influence policy decisions for greater maternal and neonatal risk reduction during pregnancy. These two findings are the effect of IPT dosing on reported malaria in this population and the effect of combined ARV therapy on HIV-1 RNA viral load. These results are not new to the scientific world, but the consistency of these findings with previous studies helps to validate the data of this pilot study.

With regards to IPT dosing, Table 3b highlights the effectiveness of 2 or more doses of IPT on reduction of reported malaria in both HIV-positive (17%) and HIV-negative populations (23%) compared to only one dose (25% and 35%) or no uptake of

IPT (62% and 42%), ($p<0.0001$, $p<0.001$). Between the two populations, though the percentage of HIV-negative subjects receiving two or more doses of IPT (31%) was higher than the percentage of HIV-positive subjects (23%), this difference was not statistically significant ($p=0.11$). In fact, these rates correlate to National Data for uptake of 2 or more doses of IPT among all Ghanaian pregnant women (30%).[16] The significant effect IPT has on reducing malaria infection in both pregnant populations is reassuring and suggests that appropriate policy is in place at present, with the emphasis on reaching more HIV-positive pregnant women. Increasing overall access to IPT, however, is another challenge.

IPT uptake also seems to be the method of malaria prevention that pregnant women will more frequently utilize in this study population, but not the only one with positive results in the HIV-positive pregnant population. Interestingly, among the HIV-positive subjects who utilized ITB, a significant percentage of subjects did not report malaria (72%, $p<0.0001$). This same effect was not seen in the HIV-negative subjects, suggesting that the use of ITN should be highly encouraged as part of routine care for HIV-positive pregnant women, perhaps to the point of giving a free bednet to every HIV-positive pregnant patient. In table 1, HIV-positive pregnant subjects were more likely to have a lower socioeconomic status based on occupation, education, and housing options, so it is possible that they are exposed more frequently to malaria than their HIV-negative counterparts. Thus, it is even more important that ITN use is encouraged during prenatal care. Overall, however, only 22% of HIV-positive subjects and 12% of HIV-negative subjects reported using ITN. Understanding the reasoning behind such low utilization may help improve future use, particularly if low utilization results from lack of access to

bednets. Providing ITNs, coupled with more aggressive IPT administration, should be the preferred antimalarial prevention strategy for HIV-positive pregnant women at Korle-Bu Hospital, and perhaps in all of Western Africa.

Though this project cannot report on MTCT rates in the research population, data from the Korle-Bu Pediatric Department reports the rate of MTCT from 2007-2009 to be less than 0.3% (unpublished). As discussed before, risk of MTCT increases 12-fold above a HIV RNA viral load of 1,000 copies/mL. The mean peripheral (5045, SD 16 014) and placental (3390, SD 10 679) HIV-1 viral loads detected in the pregnant women of this project are technically above that cut-off, but the median of both peripheral and placental HIV-1 viral loads is less than 400 copies/mL. In fact, 21 of the 28 samples (75%) for both peripheral and placental maternal blood have a HIV RNA viral load less than 1000 copies/mL. The overall low viral load based on this analysis may be responsible for the low rate of MTCT seen in the Korle-Bu Pediatric department. The low viral loads among the subjects, in return, can be attributed to basic access to care and availability of antiretrovirals at the Fevers Unit of Korle-Bu Hospital (over 60% of HIV-positive subjects received combination therapy prior to delivery and 100% received at least NVP at delivery). The significant difference in peripheral HIV viral load between subjects receiving combination therapy prior to delivery and women receiving nevirapine only at delivery supports this claim (8979 copies/ml vs 2131 copies/ml, p=0.05). Though the low rate of MTCT may suggest that further efforts to reduce risk of MTCT are not needed, this should not be the conclusion. Forty percent of HIV-positive women still only receive the most basic abbreviated ARV regimen at the biggest teaching hospital in West Africa. More efforts must be made to increase access to combination ARV prior to delivery, just

as even more PMTCT programs should be established to continue to diagnosis primigravida women who are unaware of their diagnosis. A startling 60% of women in this study were diagnosed with HIV during the current pregnancy, most likely due to increased access to testing.

Another possible reason for low rates of MTCT in this population may be due to the subtype of HIV-1 that predominates in West Africa. There are 9 major subtypes of HIV-1 (A,B,C,D,F,G,H,J,K) and 14 major recombinant forms. Epidemiological studies have shown that the different subtypes have discrete global distributions.[43] For example, subtype C accounts for more than 50% of all HIV infections and predominates in Eastern and Southern Africa, home to the worst epidemics. Eastern Africa is also home to subtype D. Subtype A, on the other hand, is found in West Africa. Several studies have suggested that subtypes C and D have higher virulence than subtype A, as subjects with subtype C and D developed AIDS faster than subjects with subtype A.[44, 45, 46] Recent studies have also investigated the association of subtype on MTCT, with heterogenous results. The majority showed no association between subtype and rates of MTCT, but two studies in East Africa (Kenya and Tanzania) found that subtype A was less transmissible than either subtype C or D.[47, 48] Therefore, it is possible that subtype is having an effect on the low rates of MTCT of HIV-1 among the subjects in this study, but many recombinant subtypes may exist in this population, therefore further research would need to be done to comment on this relationship. If the HIV subtypes predominating in the Accra area are low virulence, this may possibly explain the 18% rate of discordance among the HIV-positive pregnant women and their partners. (Table 2) Again, further research is needed to comment on this relationship as well. Determining specific subtype

distribution among the HIV-positive pregnant population could add to the understanding of another factor influencing MTCT in Ghana.

Without analysis of the placental histopathology for malaria parasitemia presence, this study cannot definitively speak to the relationship between placental malaria parasitemia and HIV-1 viral concentration, both peripheral and placental. Determining placental parasitemia presence, prevalence, and density in the HIV-positive subjects, according to histopathological analysis, would further identify the role of malaria in pregnancy among Ghanaian women, specifically its effect on MTCT. Examining the local cytokine environment in the placentas of the HIV-positive subjects in this study is also an important next step. Particularly in this population of low transmitters, cytokine profiles would be very insightful to the interplay between placental malaria and HIV transmission. Does it protect or promote MTCT? Is there placental breakdown or, in this specific population, does placental malaria parasitemia reduce risk of MTCT of HIV, as found by Ayisi et al. [37]

Though recent studies have discounted the role of increased anti-VSA IgG as a protective factor against the adverse maternal and neonatal outcomes of pregnancy-associated malaria, assessing malaria immunity within this population may be interesting. The study found no significant difference in gravity or parity between women who reported malaria and women who did not report malaria during their pregnancy, irrespective of their HIV-status. While this correlates to previous studies on HIV-positive subjects, it is contrary to studies on HIV-negative women. It is possible that pregnant Ghanaian women in this population have a high baseline level of immunity to malaria that nullifies the effect of anti-VSA IgG development or that other factors are at play,

perhaps local cytokine profiles. Assessing levels of this antibody among both HIV-positive and HIV-negative women could provide further understanding of the role of malaria in pregnancy in this population.

Whether malaria co-infection increases MTCT of HIV is uncertain in this study, but the study clearly shows that proper HIV care, access to combination antiretroviral therapy prior to delivery, and protection from malaria infection in pregnancy with ITN and 2 or more doses of IPT is of great importance to overall maternal health and reduced vertical transmission of HIV. Efforts must not stand still with regards to providing care to HIV-positive pregnant women in this population.

Limitations, Challenges, and Reflections

The project was a collaboration between the OB-GYN Department, the Fevers Unit, the Virology Department, the Microbiology Department, and the Pathology Department at Korle-Bu Hospital, as well as contributions from The Noguchi Institute and Yale School of Medicine. The collaboration was an impressive multidisciplinary undertaking in a resource-limited environment that lacked strong intradepartmental research infrastructure and history. Though, in practice, communication between all departments could have been more extensive, it laid the groundwork for future multidisciplinary research projects.

There were several limitations to this study that could have affected outcome. The first group of limitations involved the research population. The low prevalence of HIV among pregnant women in Accra, Ghana provided a small population to assess, even over a two-year period. The low numbers of HIV-positive pregnant women limited the power of the study, especially with the high attrition rate of subjects. In addition, the

timing of the project initiation unfortunately coincided with the dry season in Accra (October to April) and not the rainy or post-rainy seasons (May to September). It is possible that the number of pregnant women reporting malaria in this study would have increased if subjects had been enrolled during the rainy season, thus possibly changing the results of this study, particularly for the higher socioeconomic HIV-negative population, who would have been more exposed to malaria.

The second category of limitations, and the major challenge in this study, was the limited research budget, and the aspects of the protocol affected by it.

Dedicated research personnel were lacking on this project, specifically a dedicated translator, labor nurse, parasitologist, OB-GYN resident, and pathologist to assist in the collection and processing of data.

Translation

Not having a consistent translator available on the research project meant that people unfamiliar with the objectives of the research project or the information to be obtained were occasionally used. Significant recall and reporting bias could be affecting the current data.

Incorrect information from the subjects most likely resulted on many occasions due to language and education barrier with the subjects and translators. A dedicated translator would also be able to assist in follow-up of non-English speaking subjects after recruitment and during labor.

Labor Nurse

In a project that requires analysis of placental architecture and thick film microscopy of fresh placental blood, the timing of placental processing is extremely

important. But spending every minute on the labor floor waiting for that placenta is infeasible. Having a dedicated labor nurse or team of labor nurses paid to collect and immediately process placentas from HIV-positive pregnant women delivering at Korle-Bu would have increased placental numbers and increased quality of thick film preparation of placental blood for microscopy. Though the labor nurses in this project received a small stipend for their assistance in procuring HIV-positive placentas, the inexperience with research protocol and infrastructure prevented effective contact with the main research team when an HIV-positive pregnant woman was ready to deliver. At the beginning of the project, there was a high attrition rate of saving the placentas by the nurses, but further contact with the labor floor improved those numbers. If more funds were available, training could be offered to nurses interested in assisting in the research project for a small stipend, widespread knowledge of the project could be conveyed, and the protocol could operate more effectively and efficiently. A labor nurse would also allow the procurement of HIV-negative placentas matched for age, gravity, parity, gestational age, and mode of delivery, allowing a true comparison of placental architecture and cytokine environment between HIV-positive and HIV-negative pregnancies affected by malaria. This was very difficult to accomplish without nurse assistance on the labor floor, and thus it was abandoned early in the project.

Parasitology

The art of the thick film is multilayered, requiring just the right amount of blood, just the right thickness. It also requires high quality Giemsa stain and a dedicated parasitologist to stain and read the slides. On the streets of Accra, it is not uncommon to find fake Giemsa stain with the effectiveness of blue dye. Unfortunately, many of the

slides in this study were stained with this low quality Giemsa. In any malaria study, perfecting the procedure for thick film preparation and staining is a necessity, but it is also important to have proper training in this, particularly for a researcher unfamiliar with this process.

OB-GYN Resident

Though we attempted to utilize the OB-GYN residents as best we could for infant blood draws and obstetric chart clarifications, having a dedicated OB-GYN resident as a research assistant would help in catching inconsistencies of information reported in the chart (on several occasions, IPT doses had not been documented but the resident remembered prescribing) and in difficult infant blood draws. A dedicated OB-GYN resident could also assist in identifying both HIV-positive and HIV-negative subjects and to inform the rest of the department of ways to assist in the research project. The resident could also help in 6 month follow-ups for all subjects who delivered at Korle-Bu to assess maternal health, malaria infection postpartum, and infant HIV-conversion. This additional data would be very useful, as the effects of pregnancy-associated malaria can persist 60 days postpartum.

Due to budget restrictions, thick films to test for baseline malaria parasitemia were not performed at routine ANC visits. If a women complained of malaria symptoms, she received treatment without documentation of parasitemia. Nearly 35% of all pregnant women hospitalized for malaria did not have documented thick film assessment, nor blood hemoglobin levels (data not presented). Having a dedicated OB-GYN assistant familiar with the system to review charts and follow-up on missing information would have provided even more data to confirm malaria parasitemia and assess its affect on

maternal health.

The last category in this section involves the challenges of conducting a research project in a resource-limited setting on a tight research budget, without any prior experience. Challenging is the appropriate word. In addition to the budget constraints and language barrier, the lack of supplies to process the few samples obtained, and the complications of integrating into a foreign hospital infrastructure, the initial unfamiliarity with Ghanaian culture stood as the most significant barrier to effective implementation of the protocol. I say this because once familiar with the ways of being Ghanaian, assistance can readily be found. Of course, it took me 7 of my 8 months to become adept at Ghanaian culture, to learn the basics of Twi, the hierarchy of the hospital, the trick to infant blood draws, and the way to ensure that placentas from all HIV-positive women delivering at Korle-Bu are saved.

Research in developing nations such as Ghana remind me of the saying, "It may not be the best way or the right way, but it works." Using broken needles to draw blood from the tiny veins of newborn babies and collecting that blood drip by drip in a EDTA tube may not be the best way, but it works when butterfly needles are scarce. Reusing gloves to process samples over the course of a week is not ideal, but when you need the new ones for blood draws on the wards, it works. I am thankful to have experienced that version of medicine, a version that is a reality for the majority of people in this world. I am thankful to have begun the process of further research into the effect of malaria on HIV-positive pregnant women in Accra, Ghana, to have seen, first hand, the vulnerability of that population. I am thankful to be a part of research efforts to better understand the mechanisms impacting outcome of HIV-positive pregnancies, to better protect this

population through increased access to care, and to stop pregnancy from being the “most dangerous thing a woman can do” throughout this world.

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